Anthralin Decreases Keratinocyte TGF-α Expression and EGF-Receptor Binding In Vitro

Alice B. Gottlieb, Lakshmi Khandke, Jeffrey F. Krane, Lisa Staiano-Coico, Robin Ashinoff, and James G. Krueger

Laboratory for Investigative Dermatology, The Rockefeller University, New York; and Department of Surgery (LS-C), The New York Hospital, New York, New York, U.S.A.

Anthralin is an effective topical treatment for active psoriasis; however, its mechanism of action is unknown. Both TGF-α and its receptor, the EGF receptor, are overexpressed in active psoriatic plaques and might, therefore, play a role in psoriatic epidermal hyperplasia. In order to assess whether anthralin might act via alteration of this growth factor pathway, we examined the in vitro effects of pharmacologic concentrations of anthralin on cultured normal human keratinocytes. Keratinocyte proliferation was inhibited by 98% at an anthralin concentration of 10 ng/ml. In contrast, lymphocyte proliferation was inhibited by only 50% at an anthralin concentration of 10 μg/ml. Anthralin treatment did not induce cell-cycle–specific growth arrest as assessed by flow-cytometric analysis of acridine-orange–stained keratinocytes. Northern analysis of anthralin-treated keratinocytes demonstrated a marked decrease in TGF-α mRNA expression. Anthralin-treated keratinocytes showed decreased binding of ^{125}I-EGF and ^{125}I-IGF-I to their respective receptors, but EGF receptor binding was inhibited to a greater extent. Anthralin decreased ligand-binding affinity and cell-surface numbers of EGF receptors as assessed by Scatchard analysis of ^{125}I-EGF binding to anthralin-treated keratinocytes. These results indicate that anthralin alters components of the EGF receptor pathway in cultured keratinocytes and that these effects might contribute to the clinical efficacy of anthralin in the treatment of active psoriasis. *J Invest Dermatol 98:680–685, 1992*

Psoriatic lesions show evidence of both immune activation and epidermal growth activation. The immune activation of psoriasis resembles that seen in delayed type hypersensitivity reactions as shown by the presence of activated, IL-2 receptor + T cells and γ-interferon–induced proteins such as HLA-DR, IP-10, and ICAM-1 [1–12]. The keratinocytes of active psoriatic plaques share many features of growth activation with normal epidermis undergoing regenerative maturation [12–15]. Psoriasis thus combines epidermal hyperplasia with features of local immune activation, processes that may be regulated by established cytokines [2,10–12, 16–18].

Several cytokines and cytokine receptors are expressed at high levels in psoriatic epidermis. Both transforming growth factor-α (TGF-α) [17,19] and its receptor the epidermal growth factor (EGF) receptor [20] are overexpressed in psoriasis. Increased cutaneous and plasma levels of IL-6 have also been demonstrated in patients with active psoriasis [18,21,22]. The potential pathogenic significance of these findings is emphasized by the fact that both TGF-α and IL-6 promote keratinocyte proliferation in vitro [18,23–26]. In addition to increased EGF receptor levels, it has recently been noted that insulin-like growth factor-I (IGF-I) receptor expression appears to be increased in psoriatic epidermis [27,28]. Activation of IGF-I receptors in cultured keratinocytes increases the number of EGF receptors on the keratinocyte cell surface and also promotes keratinocyte proliferation in synergy with EGF/TGF-α [27]. Thus, the epidermal hyperplasia seen in psoriasis may be influenced both by increased cytokine expression and by increased cytokine receptor levels.

Although anthralin has been used to treat plaque-type psoriasis for many years, its mechanism of action is incompletely understood.

Manuscript received November 20, 1991; accepted for publication December 26, 1991.

This work was supported in part by General Clinical Research Center Grant MO1-RR-00102 from the National Institutes of Health to the Rockefeller University Hospital; by a Training grant (5 T32 AR07525) from the National Institutes of Health to the Laboratory for Investigative Dermatology; by the Skin Disease Society; by the Dermatology Foundation's 1989 National Psoriasis Foundation Research grant (ABG); by Marion Merrell Dow Pharmaceuticals and the Dermatology Foundation (LK); by the Thomas B. Fitzpatrick Research Award from the KAO Corporation of Japan (JGK); by a grant from Ms. Susan Weil; by a grant from Sandoz Corporation; by NIH grants GM 26145, GM 42461 and a grant from the New York Fire Fighters Skin Bank to LS-C; by a Medical Scientist Training Program grant GM 07739 to JFK; by a Training Grant from the National Institutes of Health (5 T32 AR071990) to RA, by the Carl Herzog Foundation (ABG), and with general support from the Pew Trusts.

Reprint requests to: Dr. Alice B. Gottlieb, Laboratory for Investigative Dermatology, The Rockefeller University, 1230 York Avenue, NY, NY 10021-6399.

Abbreviations:
AO: acridine orange
BB: binding buffer
CSA: cyclosporine
DMSO: dimethyl sulfoxide
EGF: epidermal growth factor
IGF-I: insulin-like growth factor-I
IL: interleukin
KBM: keratinocyte basal medium
K_d: binding dissociation constant
KGM: keratinocyte growth medium
PHA: phytohemagglutinin
PBMC: peripheral blood mononuclear cells
TGF-α: transforming growth factor-α

0022-202X/92/$05.00

Anthralin (1,8,dihydroxy-9-anthrone) contains an anthracene unit with two hydroxyl groups at positions 1 and 8 and a keto group at C-9. In aqueous solutions, light exposure catalyses auto-oxidation of anthralin with the formation of free radicals, leading to the inactive final compound, danthrone, and anthralin dimer [29]. Anthralin treatment has been reported to inhibit granulocyte function, inhibit the lipoxygenase pathways, inhibit DNA replication and repair, inhibit mitochondrial respiration, and decrease calmodulin activity in vitro [29–38]. In vivo, anthralin decreases plasminogen activator levels in plaques, inhibits mitochondrial ATP synthesis, and normalizes keratin expression [29,37–39]. However, it is unclear how anthralin treatment affects the growth factor/cytokine pathways that might regulate epidermal hyperplasia in psoriatic plaques, e.g., TGF-α/EGF-receptor, IGF-I/IGF-I receptor, and the IL-6/IL-6 receptor.

In this study, we examined the effects of anthralin on human keratinocyte growth in vitro and on components of the EGF receptor and IGF-I receptor growth pathways in vitro. Anthralin inhibited keratinocyte growth far more than lymphocyte growth, at similar pharmacologic concentrations where cell viability was not impaired. No cell-cycle–specific growth arrest was observed in anthralin-treated keratinocytes. Anthralin-treated keratinocytes showed large reductions in TGF-α expression and EGF receptor binding, whereas IGF-I receptor binding was decreased but to a lesser extent.

MATERIALS AND METHODS

Cell Proliferation Assay Keratinocytes were grown in serum-free MCDB 153 medium supplemented with epidermal growth factor 0.1 ng/ml, insulin 5 μg/ml, hydrocortisone 0.5 μg/ml, calcium 0.15 mM, bovine pituitary extract, and with gentamycin and amphotericin B (KGM; Clonetics, San Diego, CA) [12,18,23,27,40]. Anthralin (dithranol, 1,8-dihyroxy-9[1OH]-anthracenone; Sigma Chemical Company, St. Louis, MO) was diluted in dimethyl sulfoxide (DMSO) to a stock concentration of 10 mg/ml. The stock was diluted in DMSO to prepare various concentrations of anthralin. Control cultures received appropriate dilutions of DMSO alone.

Normal human keratinocytes were obtained from neonatal foreskins after dermal-epidermal separation was achieved by a 12-h incubation in 1% dispase at 4°C, followed by trypsinization for 30 min at 37°C. Keratinocytes were used for experiments after the second or third passage. Keratinocytes were cultured in T25 flasks (Corning, NY) at a density of $0.5–1 \times 10^5$ cells/flask in KGM. The keratinocytes were incubated with various concentrations of anthralin or vehicle alone for 5 d. Keratinocyte cell counts were measured using a Coulter Counter (Coulter Electronics, Inc., Hialeah, FL). Cell viability was assessed by trypan blue dye exclusion.

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized human blood by Ficoll density gradient centrifugation as previously described [41]. Various concentrations of anthralin or vehicle were added to PBMC cultured at a concentration of 1×10^6/ml for 4 d in 10 ml RPMI 1640-10% FBS (fetal bovine serum) plus glutamine and penicillin/streptomycin in the presence and absence of phytohemagglutinin (PHA; Wellcome Diagnostics, Temple Hill Dartford, U.K.) at a 1/250 dilution of the stock solution. After 4 d, cell counts were measured using a Coulter Counter. Cell viability was assessed by trypan blue dye exclusion.

Normal human neonatal foreskin fibroblasts were grown in the presence or absence of FBS as previously described [12,42].

Flow Cytometry Keratinocytes used for cell-cycle analysis were grown in serum-free media and then cell-cycle distribution was determined using flow-cytometric analysis of acridine-orange (AO)–treated keratinocytes as previously described [43,44]. Under these conditions, interactions of the dye with DNA resulted in green fluorescence with a maximum emission of 530 nm (F530), whereas interaction of the dye with RNA gave red metachromasia at 640 nm (F640). The intensities of these reactions are proportional to the DNA and RNA content, respectively.

RNA Isolation and Analysis Keratinocyte cultures were exposed to various concentrations of anthralin for 24 h. The cells were harvested and the RNA was extracted with acid guanidine thiocyanate-phenol-chloroform [45]. RNA concentration was determined by absorption at 260 nm. Equal quantities of RNA were size fractionated by electrophoresis in 1% agarose and then transferred to nitrocellulose or Genescreen (New England Nuclear, Boston, MA) by following standard protocols [45]. A full-length cDNA clone for TGF-α [25] was obtained from Dr. R. Derynck. The hybridization probe was made by random priming with ^{32}P dCTP using the random-primed DNA labeling kit obtained from Boehringer Mannheim (Indianapolis, IN). A 40-base synthetic oligonucleotide of human beta-actin (Oncogene Science, Manhasset, NY) was 5′ end labeled with γ [^{32}P]-ATP using T4 polynucleotide kinase (New England Biolaboratories, Beverly, MA). A c-myc specific dDNA probe was a generous gift from Dr. Wilson Miller (Sloan Kettering Memorial Hospital, NY, NY [46]). Hybridization was carried out at 45°C under high stringency conditions using 50% formamide. The membranes were washed and developed on Kodak XAR film at −70°C using Cronex intensifying screens.

Receptor Binding Studies Both EGF receptor and IGF-1 receptor binding assays were done as previously described [20,27,45]. Briefly, cultured human neonatal foreskin keratinocytes were grown in KGM to approximately 75% confluence in 24-well tissue culture plates. Cells were then incubated in KGM alone or containing anthralin (0.01 to 10 μg/ml) for 24 h. The plates were then washed 2×90 min at 37°C with keratinocyte basal medium (KBM; Clonetics Corporation, San Diego, CA). Plates were then put on ice and washed once with cold binding buffer (BB: KBM + 5 mg/ml BSA + 20 mM HEPES buffer, pH 7.3) followed by the addition of 0.25 ml/well of 0.05 to 5 ng/ml ^{125}I-EGF (Amersham; Specific Activity 100 μCi/μg) or 0.05 ng/ml ^{125}I-IGF-I (Amersham; Specific Activity 2000 Ci/mmol)in BB to each well. Plates were transferred to a rocking incubator at 4°C for 6 h and then washed four times with BB. Cells were lysed in 0.1 M NaOH/0.1% TX-100 and counts were read in a γ counter. Nonspecific binding was measured with a 5000-times excess of unlabeled EGF or IGF-1 (PeproTech, Rocky Hill, NJ) and did not exceed 15% of total binding. Each value represents mean specific binding of triplicate measurements. Scatchard analysis was done in the absence or presence of anthralin at a concentration of 1.0 μg/ml.

RESULTS

Anthralin Treatment Inhibits Keratinocyte Growth In Vitro Because psoriatic plaques are characterized by both an activated keratinocyte and T-lymphocyte compartment [10–12], the effects of anthralin on keratinocyte and lymphocyte growth were assessed using cultured normal human keratinocytes and lymphocytes in the presence of varying concentrations of anthralin. These experiments demonstrated that keratinocytes were much more sensitive in vitro to the inhibitory effects of anthralin on cell proliferation than were lymphocytes (Fig 1). Anthralin at concentrations of 10 ng/ml to 10 μg/ml was added to actively proliferating foreskin keratinocytes grown under optimal serum-free conditions (Fig 1*A*) and to normal lymphocytes stimulated with the mitogen, PHA (Fig 1*B*). At concentrations of 10 ng/ml, anthralin caused a 98% inhibition of keratinocyte proliferation. In contrast, even at anthralin concentrations of 10 μg/ml, only 50% inhibition of mitogen-induced lymphocyte proliferation was observed. Keratinocyte and lymphocyte viabilities were greater than 99% at concentrations of anthralin less than 10 μg/ml; therefore, cell death could not account for anthralin's marked inhibition of keratinocyte growth. These concentrations of anthralin are much less than those achieved in the epidermis of patients treated with 0.1% anthralin in vivo. For example, the epidermal level of anthralin that results from the application of a 0.1% preparation is 93.66 μM [29]. A 1 μg/ml solution of anthralin is equivalent to a 4.4 μM solution. The concentrations used for the experiments described in this paper are within those achieved in epidermis after routine clinical use, but the intraepidermal distribution of anthralin after topical administration is unknown.

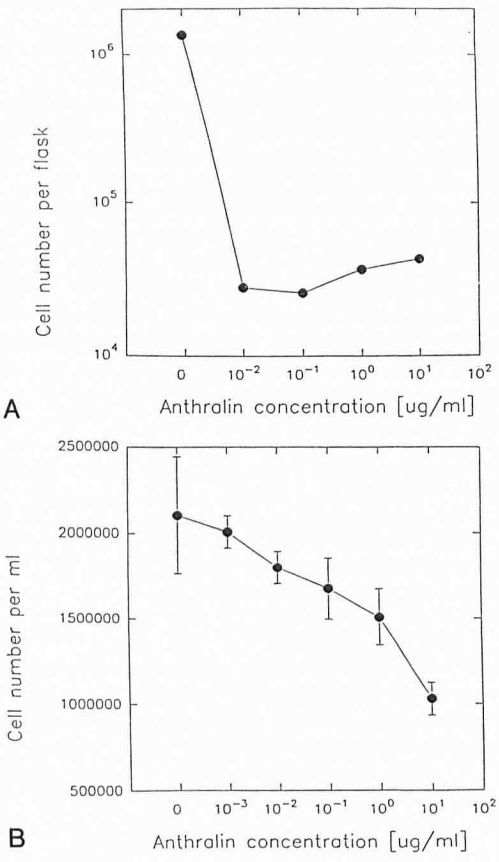

Figure 1. Keratinocytes are more sensitive to the growth-inhibitory effects of anthralin than are lymphocytes. Anthralin (0.01 – 10 μg/ml) was added to normal human foreskin keratinocytes grown in KGM for 5 d *(A)* and to PHA-stimulated PBMC cultured for 4 d *(B)*. Cell counts were determined using a Coulter Counter.

In order to determine if anthralin inhibited keratinocyte proliferation by inducing cell-cycle specific arrest, anthralin-treated keratinocytes were labeled with acridine orange and then subjected to flow-cytometric analysis as we have previously described [43,44]. Using this technique, both the RNA and DNA contents of individual keratinocytes can be assessed simultaneously. Previous studies have demonstrated that cultured keratinocytes can be divided into three populations that differ in their RNA content and proportion of cells, which are actively dividing [43,44]. Keratinocytes cultured in the presence of anthralin from 0.01 to 1.0 μg/ml showed no significant difference in the percentage of cells in G_0/G_1, and G_2 + M phases of the cell cycle (Table I). However, the RNA content of keratinocytes treated with 0.1 μg/ml of anthralin decreased as demonstrated by the reduced "high RNA" peak at 640 nm (Fig 2). This decrease in cellular RNA primarily represents a decrease in ribosomal RNA. In separate experiments, the addition of anthralin did not diminish the accumulation of the rhodamine analog, rhodamine 123 [47], into the mitochondria of viable keratinocytes (data not shown). Because anthralin treatment decreased ribosomal RNA content but did not inhibit mitochondrial-specific dye uptake, anthralin has differential effects on growth-related cellular organelles.

Anthralin Treatment Inhibits Keratinocyte TGF-α Expression In Vitro TGF-α promotes keratinocyte growth [25] and it and the EGF-receptor are overexpressed in psoriatic keratinocytes [17,19,20]. The effect of anthralin, which inhibits keratinocyte proliferation, on TGF-α expression was next studied. Addition of anthralin inhibited TGF-α expression and decreased EGF receptor binding.

Table I. Anthralin Treatment Does Not Cause Specific Cell Cycle Arrest

	Cell-Cycle Distribution (%)		
Anthralin (μg/ml)	G_0/G_1	S	G_2/M
Control	41.2	40.6	18.2
0.01	37.4	49.4	13.2
0.10	34.3	38.7	27.0
1.0	43.9	41.2	14.9
10.0	44.1	27.1	28.8

Northern analysis was performed under high stringency conditions on actively proliferating keratinocytes grown in the presence of anthralin (0.01 to 10 μg/ml) for 24 h (Fig 3). A single mRNA band was observed at approximately 4 kilobases as has been previously reported [45]. Addition of anthralin at concentrations of 0.1 to 10 μg/ml caused a dramatic decrease in TGF-α mRNA expression. The total amount of RNA applied to each lane was identical as shown by ethidium bromide staining of total RNA. To control for effects of anthralin to globally decrease mRNA production, blots were re-probed for actin mRNA. Whereas less actin specific mRNA was observed with increasing anthralin concentrations, TGF-α-specific mRNA diminished at a faster rate for each anthralin concentration studied. For example, at 0.1 μg/ml anthralin, densitometric scanning demonstrated a reduction in TGF-α mRNA expression to only 8% of that of untreated keratinocytes, yet actin mRNA remained at 67% of that of untreated keratinocytes.

In order to determine if anthralin non-specifically decreased the expression of another growth-associated gene, the effect of anthralin addition (0.01 to 10 μg/ml) on keratinocyte c-myc mRNA expression was next tested (Fig 4). Transcription of c-myc is inducible in fibroblasts by serum exposure [48] and is constitutively expressed in human keratinocytes at relatively high levels [49,50]. Figure 4 *(lanes 1–4)* shows induction of c-myc in growth-arrested human

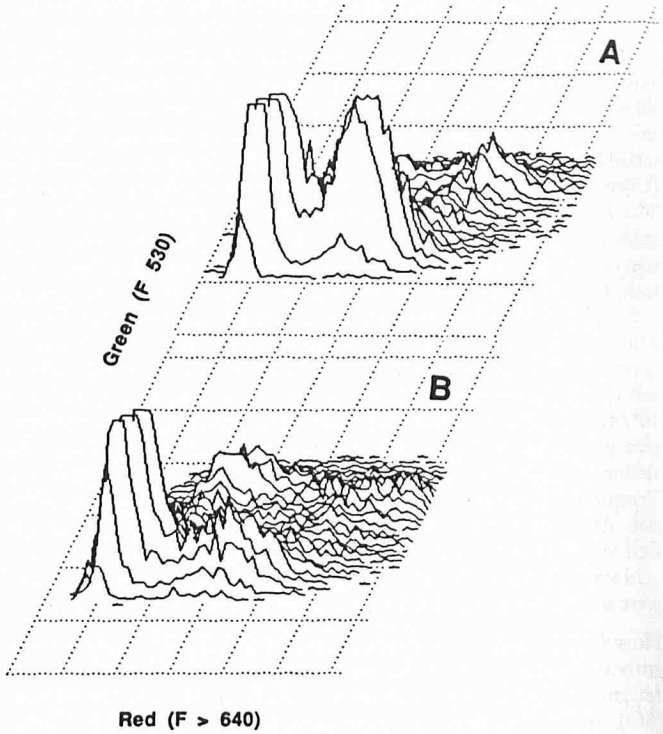

Figure 2. Anthralin decreases total cellular RNA in cultured keratinocytes. Flow cytometric analysis of acridine orange-stained control *(A)* and anthralin (0.1 μg/ml)-treated *(B)* cultured keratinocytes. Fluorescence intensity at 640 nm and at 530 nm is proportional to RNA and DNA content, respectively.

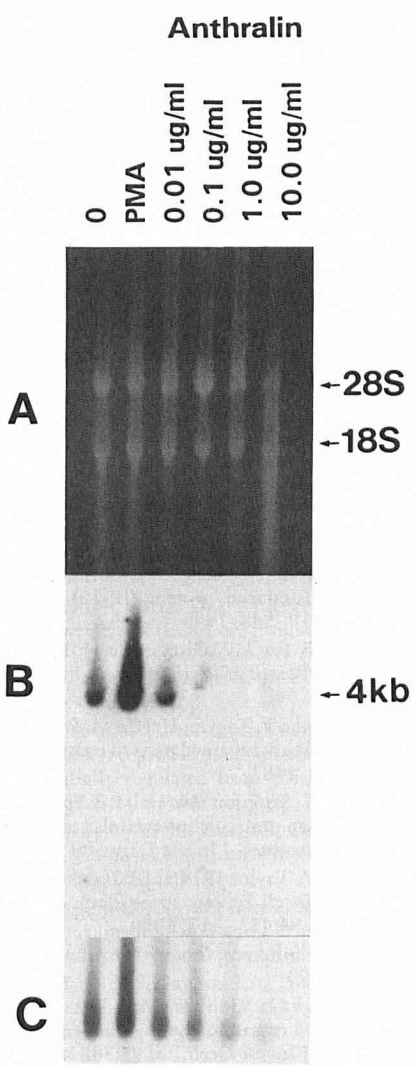

Figure 3. Anthralin inhibits TGF-α mRNA expression in cultured keratinocytes. Northern analysis was performed under high-stringency conditions on actively proliferating keratinocytes grown in the presence of anthralin (0.01 to 10 μg/ml) for 24 h. The total amount of RNA applied to each lane was identical as shown by ethidium bromide staining of total RNA *(A)*. Addition of anthralin at concentrations of 0.1 to 10 μg/ml caused a dramatic decrease in TGF-α mRNA expression *(B)*. The blot was reprobed with an actin-specific probe *(C)*. Whereas less acting-specific mRNA was observed with increasing anthralin concentrations, TGF-α–specific mRNA diminished at a faster rate for each anthralin concentration studied.

dermal fibroblasts after serum treatment in comparison to constitutive expression in human keratinocytes *(lane 5)*. Expression of c-myc mRNA was increased somewhat by treatment of keratinocytes with TPA *(lane 6)* or anthralin *(lanes 7–9)*. In comparison to expression of TGF-α mRNA or actin mRNA, c-myc mRNA was not diminished by treatment of cells with anthralin as high as 10 μg/ml.

Anthralin Decreases EGF and IGF-1 Receptor Binding

IGF-1 and EGF work in synergy to promote keratinocyte proliferation in vitro [27]. The effect of adding anthralin on the binding of EGF and IGF-1 to their respective receptors on keratinocytes cultured under optimal conditions was next investigated (Fig 5*A*). Addition of 0.1–10 μg/ml anthralin caused a sharp decrease in EGF binding to the EGF-receptor. IGF-1 binding to the IGF-1 receptor was also inhibited but to a lesser extent. The differential sensitivities of the EGF and IGF-1 receptors to anthralin's effects suggests there is specificity to anthralin's mode of action. In separate experiments, Scatchard analysis revealed that anthralin (1.0 μg/ml) decreased

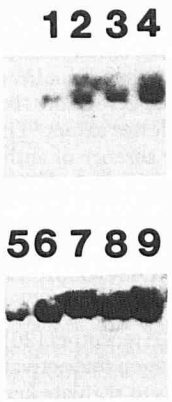

Figure 4. Anthralin does not decrease c-myc mRNA expression. C-myc mRNA expression is induced in growth arrested human dermal fibroblasts *(lane 1)*, after 10% serum treatment for 0.5 *(lane 2)*, 1.0 *(lane 3)*, and 2.0 *(lane 4)* h in comparison to constitutive expression in human keratinocytes *(lane 5)*. Expression of c-myc mRNA was increased somewhat by treatment of keratinocytes with TPA *(lane 6)* or anthralin at 0.01 *(lane 7)*, 1.0 *(lane 8)*, and 10.0 *(lane 9)* μg/ml as compared to the control *(lane 5)*.

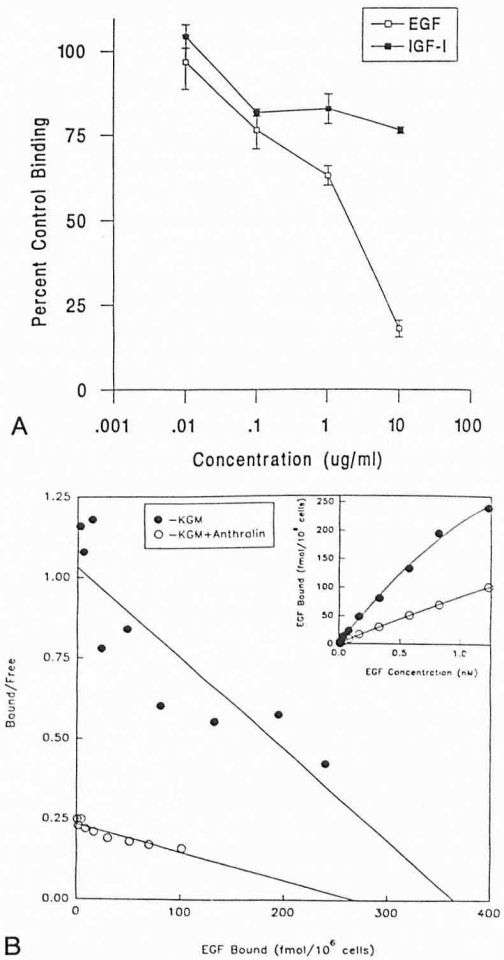

Figure 5. Anthralin decreases EGF- and IGF-1–receptor binding. The effect of adding anthralin on the binding of EGF and IGF-1 to their respective receptors on keratinocytes cultured under optimal conditions was next investigated *(A)*. Addition of 0.1–10 μg/ml anthralin caused a sharp decrease in EGF binding to the EGF-receptor. IGF-1 binding to the IGF-1 receptor was also inhibited but to a lesser extent. Scatchard analysis revealed that anthralin (1.0 μg/ml) decreased both EGF-receptor binding affinity and receptor number *(B)*.

EGF-receptor binding affinity and cell surface receptor numbers (Fig 5*B*). The binding dissociation constant (K_d) for binding of EGF to its receptor in the absence of anthralin was 0.5 nM, whereas in the presence of anthralin it was 2.1 nM. EGF receptor cell-surface numbers were decreased to a lesser extent. The number of receptors per cell was 2.1×10^5 in the absence of anthralin and 1.7×10^5 in the presence of anthralin. Thus, reduced binding of EGF to its receptor appears to be primarily mediated by diminished binding affinity.

DISCUSSION

Epidermal hyperplasia in psoriasis is strongly correlated with increased expression of EGF receptors [20] and of TGF-α [17,19], a keratinocyte-derived mitogen that activates the EGF receptor. IGF-1 receptors, which appear to regulate keratinocyte proliferative responses to EGF or TGF-α [27], also appear to be overexpressed in active psoriatic epidermis [28]. Because epidermal hyperplasia in psoriatic skin is often transient, altered epidermal growth may be primarily regulated by expression of growth factor receptors and/or the local availability of ligands for these receptors. In support of this concept, overexpression of TGF-α in epidermal tissue of transgenic mice produces focal epidermal hyperplasia, though a second stimulus may be required for extensive skin involvement [51]. Thus TGF-α, produced by keratinocytes, and EGF receptor expression by keratinocytes may be important targets of anti-psoriatic therapeutic agents.

The results of this study suggest that pharmacologically relevant concentrations of anthralin have the ability to strongly inhibit keratinocyte proliferation in vitro, possibly by affecting early steps in mitogenic signalling pathways. Anthralin diminishes ligand binding to the EGF receptor via a reduction in binding affinity. Previous work has demonstrated that EGF receptor affinity and ligand-induced tyrosine kinase activity are greatly reduced by protein kinase C activation as a result of phosphorylation of the EGF receptor on threonine residue 654 [52,53]. Anthralin may also block EGF receptor effects by decreasing binding affinity, although the mechanism of action for anthralin may be different from that of protein kinase C. Anthralin treatment significantly decreases the abundance of mRNA for TGF-α. Because TGF-α mRNA abundance is regulated in keratinocytes by activation of the EGF receptor [25], the diminished TGF-α in anthralin-treated cells could be mediated either via effects of anthralin on the EGF receptor or by direct effects on TGF-α mRNA transcription or stability. Anthralin might also diminish keratinocyte proliferation through its effects on the IGF-1 receptor [27], though the effect of anthralin on this growth factor receptor is quantitatively smaller than its effects on the EGF receptor. Other effects of anthralin on epidermal keratinocytes, including diminished respiration [38], diminished DNA synthesis [35,36], and normalization of keratin expression [37], may be secondary consequences of diminished mitogenic signaling from EGF, IGF-1, or other membrane growth factor receptor systems.

The ability of anthralin to affect EGF receptor expression and TGF-α mRNA in cultured human keratinocytes contrasts markedly with cyclosporine A (CSA), another effective anti-psoriatic therapy. Whereas CSA also directly inhibits keratinocyte proliferation, it does not significantly diminish EGF receptor expression or TGF-α mRNA production by human keratinocytes [45]. CSA induces a proliferative block in the G_1 phase of the cell cycle, whereas anthralin does not block proliferation at a single point in the keratinocyte cell cycle [45]. Thus, these anti-psoriatic compounds, which have direct epidermal effects, may have different mechanisms of inhibiting keratinocyte proliferation in vitro. In vivo, the anti-psoriatic mechanisms of anthralin and CSA may be significantly different, because lymphocyte proliferation in vitro is more strongly suppressed by low concentrations of CSA, whereas keratinocyte proliferation is more strongly inhibited by low concentrations of anthralin [45]. It is interesting that clinical treatment of psoriatic lesions with anthralin appears to produce relatively long remissions after cessation of therapy, whereas there is a predictable relapse of active psoriasis shortly after cessation of therapy with CSA [54,55]. The ability of anthralin to decrease keratinocyte mitogenic activation by TGF-α might be important in this regard, as CSA has relatively little effect on this pathway in vitro [45] or in vivo [56,57]. In preliminary studies, treatment with 0.1% anthralin in vivo decreased immunoreactive TGF-α in a treated psoriatic plaque consistent within vitro results described above. Clearly, further analysis of psoriatic tissue treated in vivo with anthralin will be needed to further clarify its mode of action, especially in comparison to other anti-psoriatic agents. Elucidation of the therapeutic mechanisms of anti-psoriatic drugs may be useful to help understand fundamental cellular alterations that cause psoriasis and may also be useful for designing better therapies.

REFERENCES

1. Gottlieb AB, Lifshitz B, Fu SM, Staiano-Coico L, Wang CY, Carter DM: Expression of HLA-DR molecules by keratinocytes and presence of Langerhans cells in the dermal infiltrate of active psoriatic plaques. J Exp Med 164:1013–1028, 1986
2. Gottlieb AB: Immunologic mechanisms in psoriasis. J Am Acad Dermatol 18:1376–1380, 1988
3. Gottlieb AB, Luster AD, Posnett DN, Carter DM: Detection of a gamma-interferon-induced protein (IP-10) in psoriatic plaques. J Exp Med 168:941–948, 1988
4. Baker BS, Swain AF, Fry L, Valdimarsson H: Epidermal T lymphocytes and HLA-DR expression in psoriasis. Br J Dermatol 11:555–564, 1984
5. Terui T, Aiba S, Tanaka T, Tagami H: HLA-DR antigen expression on keratinocytes in highly inflamed parts of psoriatic lesions. Br J Dermatol 116:87–93, 1987
6. Singer KH, Tuck DT, Sampson HA, Hall RP: Epidermal keratinocytes express the adhesion molecule intercellular adhesion molecule-1 in inflammatory dermatoses. J Invest Dermatol 92:746–750, 1989
7. Chin YH, Falanga V, Taylor JR, Cai JP, Bax J: Adherence of human helper/memory T-cell subsets to psoriatic dermal endothelium. J Invest Dermatol 94:413–417, 1990
8. Springer TA: Adhesion receptors of the immune system. Nature 346:425–434, 1990
9. Petzelbauer P, Stingl G, Wolff K, Volc-Platzer B: Cyclosporin A suppressed ICAM-1 expression by papillary endothelium in healing psoriatic plaques. J Invest Dermatol 96:362–369, 1991
10. Nickoloff BJ: The cytokine network in psoriasis. Arch Dermatol 127:871–884, 1991
11. Gottlieb AB: Immunologic mechanisms in psoriasis. J Invest Dermatol 95:18S–19S, 1990
12. Krueger JG, Krane JF, Carter DM, Gottlieb AB: Role of growth factors, cytokines, and their receptors in the pathogenesis of psoriasis. J Invest Dermatol 94:135S–140S, 1990
13. Murphy D, Smoller B, Gottlieb AB, Hsu A, Carter DM, Krueger JG: Chronic, non-healing skin ulcers display a growth-activated epidermis (regenerative maturation) with high level TGF-α and EGF-receptor expression (abstr). J Invest Dermatol 94:557, 1990
14. Krane JF, Murphy DP, Gottlieb AB, Carter DM, Hart CE, Krueger JG: Increased dermal expression of platelet-derived growth factor receptors in growth-activated skin wounds and psoriasis. J Invest Dermatol 96:983–986, 1991
15. Mansbridge JN, Knapp AM: Changes in keratinocyte maturation during wound healing. J Invest Dermatol 89:253–263, 1987
16. Gottlieb AB, Krueger JG: HLA region genes and immune activation in the pathogenesis of psoriasis. Arch Dermatol 126:1083–1086, 1990
17. Gottlieb AB, Chang CK, Posnett DN, Fanelli B, Tam JP: Detection of transforming growth factor α in normal, malignant, and hyperproliferative human keratinocytes. J Exp Med 167:670–675, 1988
18. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, May LT, Kupper TS, Sehgal PB, Gottlieb AB: Interleukin-6 (IL-6) is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA 86:6367–6371, 1989
19. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor α in psoriatic epidermis. Science 243:811–814, 1989

20. Nanney LB, Stoscheck CM, Magid M, King LE Jr.: Altered ^{125}I epidermal growth factor binding and receptor distribution in psoriasis. J Invest Dermatol 86:260–265, 1986
21. Neuner P, Urbanski A, Trautinger F, Moller A, Kirnbauer R, Kapp A, Schopf E, Schwarz T, Luger TA: Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 97:27–33, 1991
22. Oxholm A, Oxholm P, Staberg B, Bendtzen K: Interleukin-6 in the epidermis of patients with psoriasis before and during PUVA treatment. Acta Derm Venereol (Stockh) 69:195–199, 1989
23. Krueger J, Ray A, Tamm I, Sehgal PB: Expression and function of interleukin-6 in epithelial cells. J Cell Biochem 45:327–334, 1991
24. Kupper TS, Lee F, Birchall N, Clark S, Dower S: Interleukin 1 binds to specific receptors on human and keratinocytes and induces granulocyte macrophage colony-stimulating factor mRNA and protein. A potential autocrine role for interleukin 1 in epidermis (abstr). J Clin Invest 82:1787–1792, 1988
25. Coffey RJ, Jr, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR: Production and auto-induction of transforming growth factor-α in human keratinocytes. Nature 328:817–823, 1987
26. Massague J: Transforming growth factor-α. A model for membrane-anchored growth factors. J Biol Chem 15:21393–21396, 1990
27. Krane JF, Murphy DP, Carter DM, Krueger JG: Synergistic effects of epidermal growth factor (EGF) and insulin-like growth factor 1/somatomedin C (IGF-1) on keratinocyte proliferation may be mediated by IGF-1 transmodulation of the EGF receptor. J Invest Dermatol 96:419–424, 1991
28. Krane JF, Gottlieb AB, Carter DM, Krueger JG: The Insulin-like Growth Factor 1 receptor is overexpressed in psoriatic epidermis, but is differentially regulated from the epidermal growth factor receptor. J Exp Med (in press)
29. Kemeny L, Ruzicka T, Braun-Falco O: Dithranol: a review of the mechanism of action in the treatment of psoriasis vulgaris. Skin Pharmacology 3:1–20, 1990
30. Schroder JM, Kosfeld U, Christophers E: Multifunctional inhibition by anthralin in nonstimulated and chemotactic factor stimulated human neutrophils. J Invest Dermatol 85:30–34, 1985
31. Tucker WFG, MacNeil S, Dawson RA, Tomlinson S, Bleehen SS: An investigation of the ability of antipsoriatic drugs to inhibit calmodulin activity: A possible mode of action of dithranol (Anthralin). J Invest Dermatol 87:232–235, 1986
32. Wollina U, Klinger R, Wetzker R, Knopf B: Calmodulin inhibition by Anthralin? Investigations in vitro and in vivo. Dermatologica 179:61–63, 1989
33. Czernielewski J, Juhlin L, Shroot S, Brun P: Langerhans cells in patients with psoriasis: effect of treatment with PUVA, PUVA bath, etretinate and anthralin. Acta Derma Venereol 65:97–101, 1985
34. Chang A, Alkemade H, VandeKerkhof PCM: Dithranol modulates the leukotriene B4-induced intraepidermal accumulation of polymorphonuclear leukocytes. J Invest Dermatol 92:806–808, 1989
35. Lowe NJ: Anthralin. Different concentration effects on epidermal cell DNA synthesis rates in mice and clinical responses in human psoriasis. Arch Dermatol 117:698–700, 1981
36. Clark JM, Hanawalt PC: Inhibition of DNA replication and repair by anthralin and danthron in cultured human cells. J Invest Dermatol 79:18–22, 1982
37. Holland DB, Wood EJ, Cunliffe WJ, Turner DM: Keratin gene expression during the resolution of psoriatic plaques: effect of dithranol, PUVA, etretinate and hydroxyurea regimens. Br J Dermatol 120:9–19, 1989
38. Fuchs J, Nitschmann WH, Packer L: The antipsoriatic compound anthralin influences bioenergetic parameters and redox properties of energy transducing membranes. J Invest Dermatol 94:71–76, 1990
39. Lotti T, Bonan P, Cannarozzo G, Fedi AM, Panconesi E: Antipsoriatic therapies inhibit epidermal plasminogen activator activity. Intl J Dermatol 29:528–530, 1990
40. Sehgal PB: Interleukin 6 in infection and cancer. Proc Soc Exptl Biol Med 195:183–191, 1990
41. Gottlieb AB, Lahita RG, Chiorazzi N, Kunkel HG: Immune function in systemic lupus erythematosus: Impairment of in vitro T-cell proliferation and in vivo antibody response to exogenous antigen. J Clin Invest 63:885–892, 1979
42. Gottlieb AB, Mayer L, Bonetti F, Knowles DM, Krueger J, Kunkel HG, Carter DM: A membrane protein preferentially expressed by a subpopulation of immature lymphoid cells, epidermal basal keratinocytes and other epithelial stem cells. J Am Acad Dermatol 13:54–65, 1985
43. Staiano-Coico L, Higgins PJ, Darzynkiewicz Z, Kimmel M, Gottlieb AB, Pagan-Charry I, Madden MR, Finkelstein JL, Hefton JM: Human keratinocyte culture: Identification and staging of epidermal cell subpopulations. J Clin Invest 77:396–404, 1986
44. Staiano-Coico L, Gottlieb AB, Barazani L, Carter DM: RNA, DNA and cell surface characteristics of lesional and non-lesional psoriatic skin. J Invest Dermatol 88:646–651, 1987
45. Khandke L, Ashinoff R, Krane JF, Staiano-Coico L, Granelli-Piperno A, Luster AD, Carter DM, Krueger JG, Gottlieb AB: Cyclosporine in psoriasis treatment: inhibition of keratinocyte cell-cycle progression in G1 independent of effects on transforming growth factor-α/epidermal growth factor receptor pathways. Arch Dermatol 127:1172–1179, 1991
46. Miller WH Jr, Moy D, Li A, Grippo JF, Dmithrovsky E: Retinoic acid induces down-regulation of several growth factors and proto-oncogenes in a human embryonal cancer cell line. Oncogene 5:511–517, 1990
47. Darzynkiewicz Z, Staiano-Coico L, Melamed MR: Increased mitochondrial uptake of rhodamine 123 during lymphocyte stimulation. Proc Natl Acad Sci USA 78:2383–2387, 1981
48. Katan M, Parker PJ: Oncogenes and cell control. Nature 332:203–204, 1988
49. Elder JT, Klein SB, Tavakkol A, Fisher GJ, Hickoloff BJ, Voorhees JJ: Growth factor and proto-oncogene expression in psoriasis. J Invest Dermatol 95:7S–9S, 1990
50. Elder JT, Zeigler ME, Wicha S, Voorhees JJ: Increased c-myc and c-ras mRNA levels in psoriatic epidermis (abstr). J Invest Dermatol 90:555, 1988
51. Vassar R, Fuchs E: Transgenic mice provide new insights into the role of TGF-α during epidermal development and differentiation. Genes Devel 5:714–727, 1991
52. Hunter T, Ling N, Cooper JA: Protein kinase C phosphorylation of the EGF receptor at a threonine residue close to the cytoplasmic face of the plasma membrane. Nature 311:480–483, 1984
53. Davis RJ: Independent mechanisms account for the regulation by protein kinase C of the epidermal growth factor receptor affinity and tyrosine-protein kinase activity. J Biol Chem 263:9462–9469, 1988
54. Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, Cooper KD, Baadsbaard O, Duell EA, Annesley TM, Turcotte JG, Voorhees JJ: Cyclosporine improves psoriasis in a double-blind study. JAMA 256:3110–3116, 1986
55. Ellis CN, Fradin MS, Messana JM, Brown MD, Siegel MT, Hartley AH, Rocher LL, Wheeler S, Hamilton TA, Parish TG, Ellis-Madu M, Duell E, Annesley TM, Cooper KD, Voorhees JJ: Cyclosporine for plaque-type psoriasis. Results of a multidose, double-blind trial. N Engl J Med 324:277–284, 1991
56. Gottlieb AB, Grossman RM, Khandke L, Carter DM, Sehgal PB, Fu SM, Granelli-Piperno A, Rivas M, Barazani L, Kreuger JG: Studies of the effect of cyclosporine in psoriasis in vivo: combined effects on activated T lymphocytes and epidermal regenerative maturation. J Invest Dermatol 98:302–309, 1992
57. Gottlieb AB, Krueger JG, Khandke L, Grossman RM, Delaney RJ, Fu SM, Barazani L, Carter DM: Cyclosporine A treatment decreases immune activation in psoriatic plaques without decreasing keratinocyte regenerative maturation (growth activation) (abstr). J Invest Dermatol 96:570, 1991